UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

INTRODUCTION

IN-SILICO

IN VITRO

EX VIVO

IN VIVO

CONCLUSIONS

Receptor and blood-brain barrier characterization of opioid peptides in drug research & early development

M. Verbeken, S. Stalmans, E. Wynendaele, N. Bracke, K. Peremans, I. Polis, C. Burvenich and B. De Spiegeleer

Drug Quality and Registration (DruQuaR) group

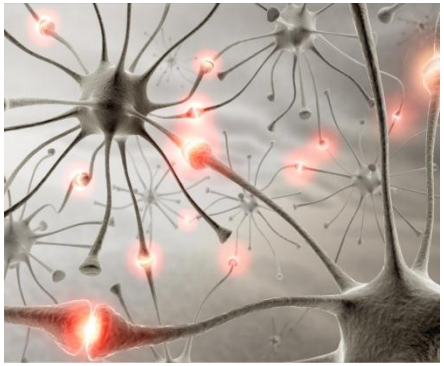
O. Ref.: 2012-239e

" Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. (The International Association for the Study of Pain)"

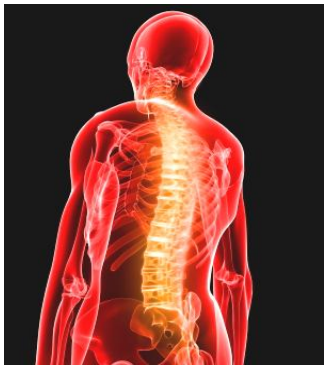
Three classes of pain:

Nociceptive

Neuropathic



Inflammatory



! Opioid peptides are key role players in modulation of pain !

→ Endogenous + externally administered

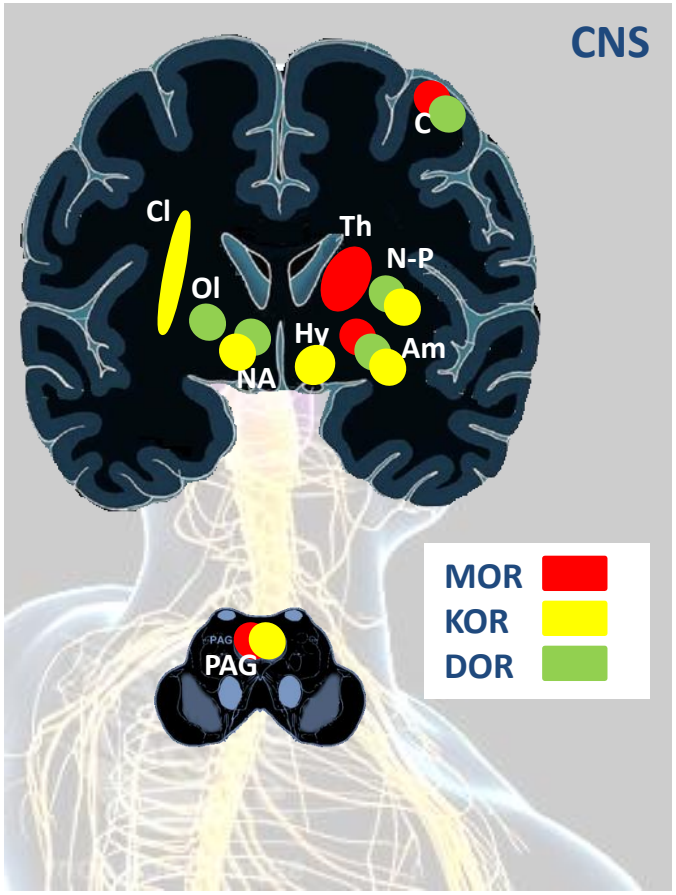
+ other systems (*e.g.* CB)

Ref. *J.D. Loeser, R. Melzack, Lancet, 353 (1999) 1607-1609.*
C.J. Woolf, J Clin Invest, 120 (2010) 3742-3744.

UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

Localization of opioid receptor expression



MOR: μ_1, μ_2, μ_3

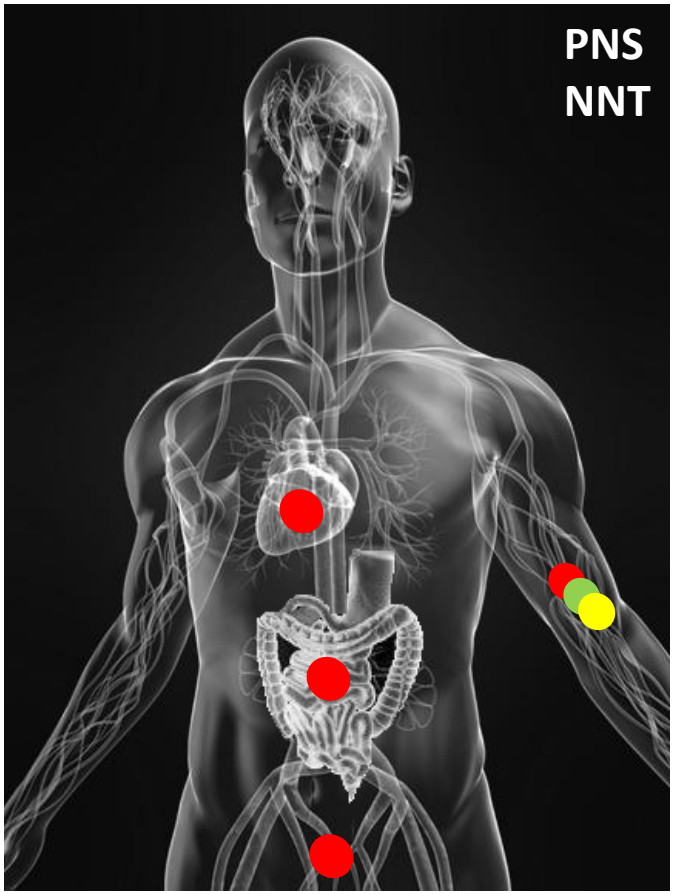
KOR: κ_1, κ_2

DOR: δ_1, δ_2

NOR: ORL_1

OPIOID PEPTIDES
WIDE RANGE OF PHARMACOLOGICAL RESPONSES:

Pain and analgesia
Stress and anxiety
Tolerance and dependence
Learning and memory
Eating and drinking
Endocrinology
Mental illness and mood
Neurological disorders
Neurophysiology
Gastrointestinal, renal and hepatic functions
Cardiovascular
Immunological

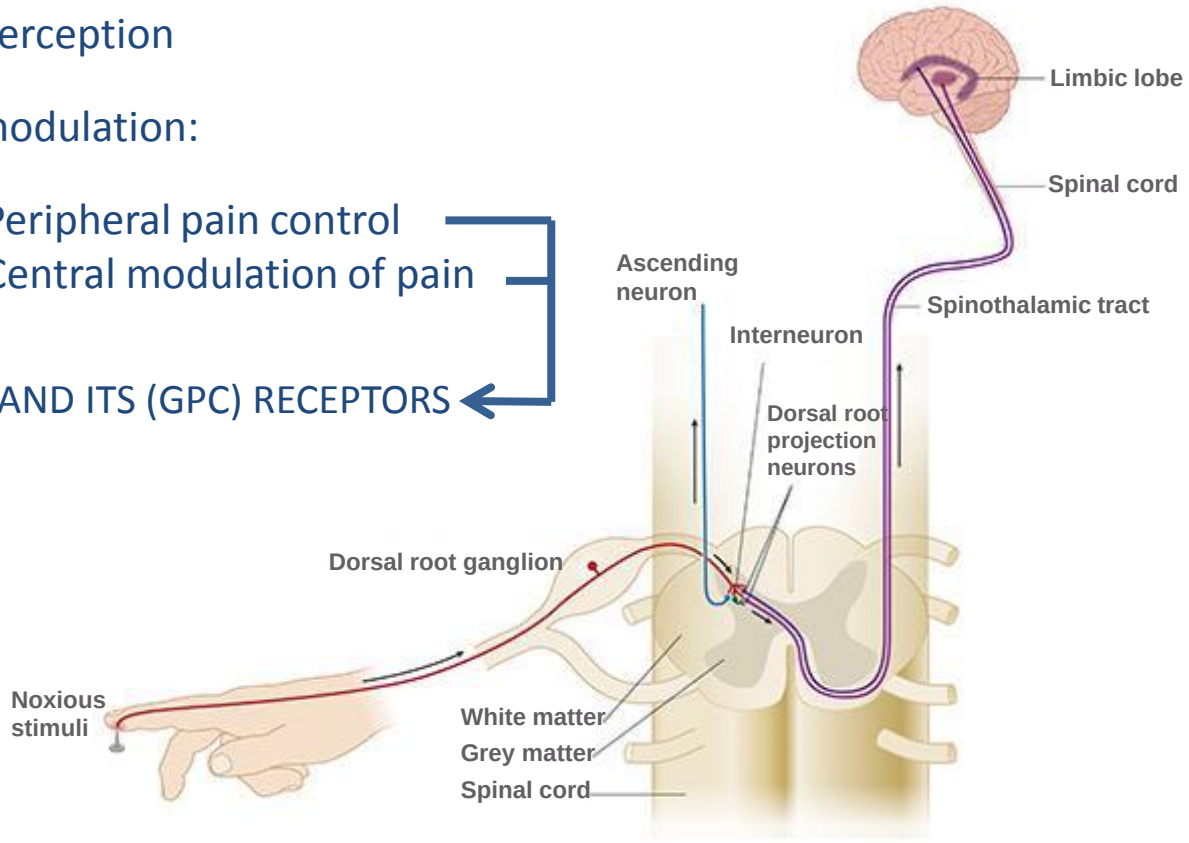


Ref. *A. Lesniak, A.W. Lipkowski, Acta Neurobiol Exp, 71 (2011) 129-138.*
P.G. Fine , K.P. Russell KP (2004), A Clinical Guide to Opioid Analgesia (Chapter 2), McGraw Hill.

Pain process:

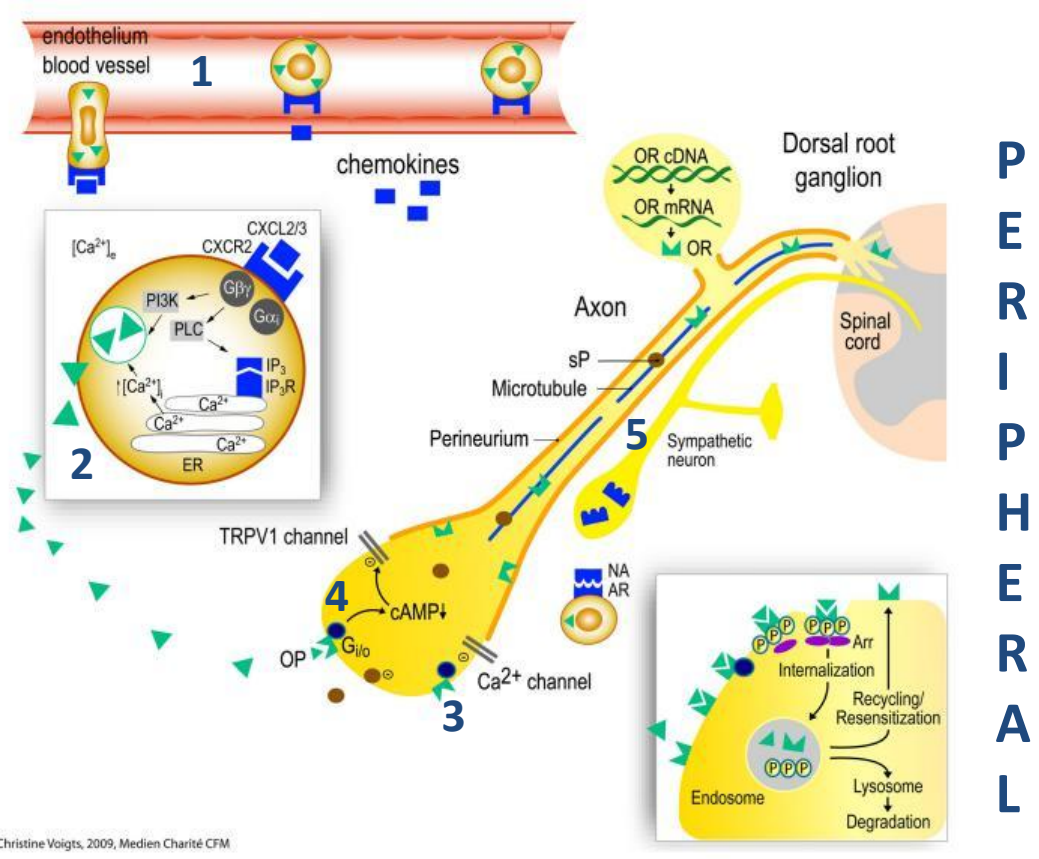
- Pain perception
- Pain modulation:
  - Peripheral pain control
  - Central modulation of pain

OPIOIDS AND ITS (GPC) RECEPTORS

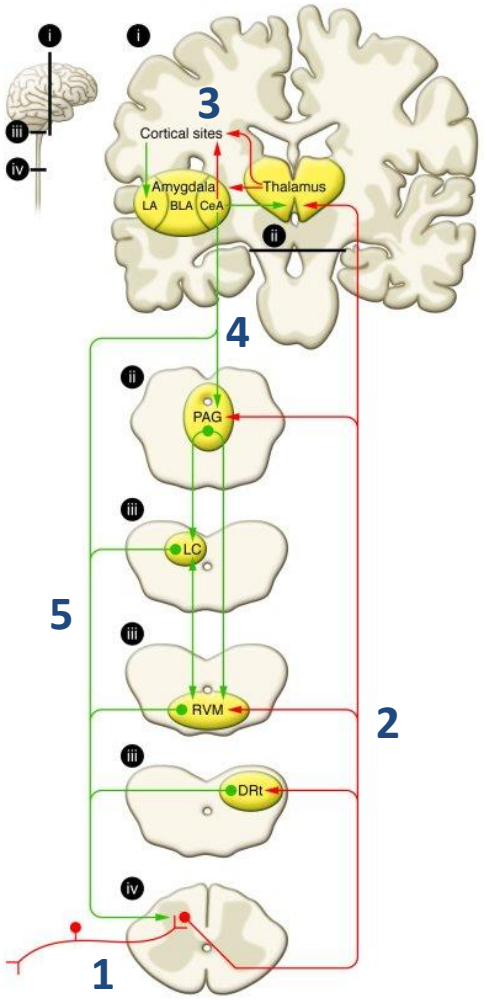


Ref. *M.H. Ossipov, G.O. Dussor, F. Porreca, J Clin Invest, 120 (2011) 3779-3787.*
N. Sehgal, H.S. Smith, L. Manchikanti, Pain Physician, 14 (2011) 249-258.

Role of opioid peptides in pain modulation:



PERIPHERAL



CENTRAL

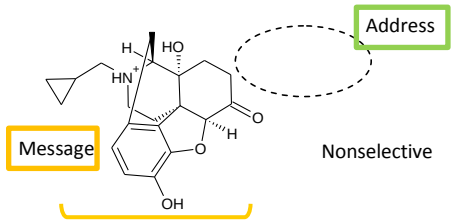
Ref. *M. Busch-Dienstfertig, C. Stein, Brain Behav Immun, 24 (2009) 683-694.*
M.O. Urban, G.F. Gebhart, Med Clin North Am, 83 (1999) 585-596.

Molecular recognition of opioid ligand-receptor interactions: *In silico* research.

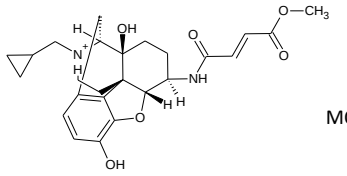
MESSAGE-ADDRESS CONCEPT

Receptor	MOR	KOR	DOR
TM3	Asp^{147} Tyr^{148}	Asp^{138} Tyr^{139}	Asp^{128} Tyr^{129}
TM5	Lys^{233} Phe^{237}	Lys^{227} Phe^{231}	Lys^{214} Phe^{218}
TM6	Val^{300} Ala^{304} His^{297} Lys^{303}	Ile^{294} Ala^{298} His^{291} Glu^{297}	Val^{281} Thr^{285} His^{278} Trp^{284}
TM7	Trp^{318} Gln^{314}	Tyr^{312} Leu^{309}	Leu^{300} Val^{296}

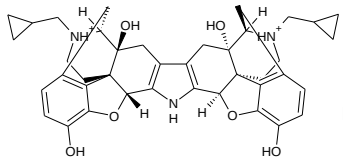
ADDRESS determines selectivity → MOR: polar interaction; KOR: salt bridge interaction; DOR: hydrophobic interaction



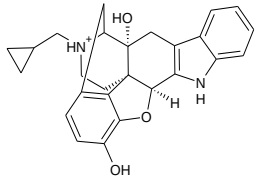
Naltrexone



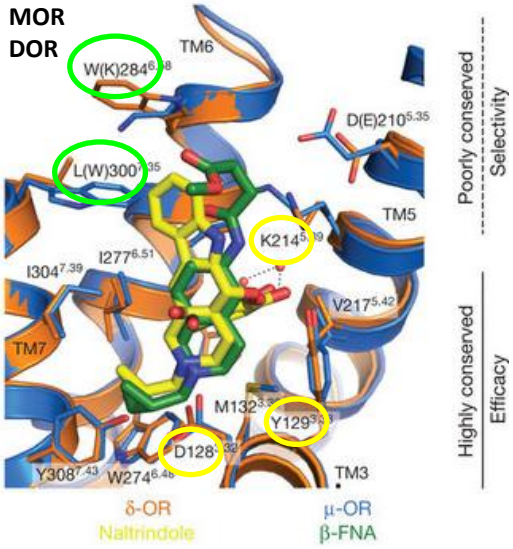
β-funaltrexamine

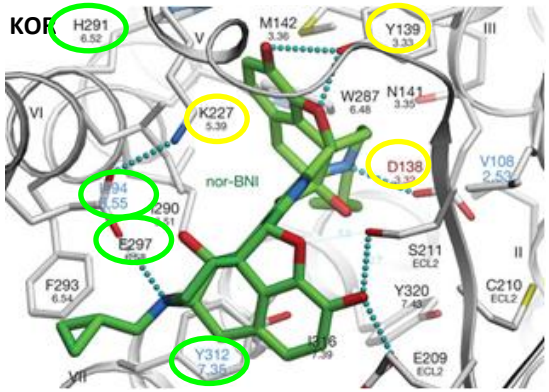


Norbinaltorphimine



Naltrindole

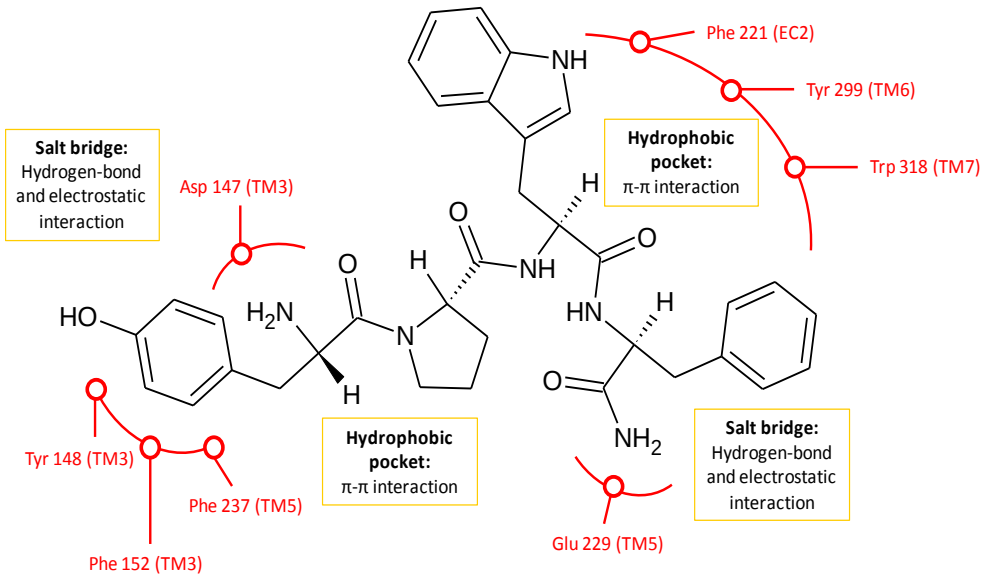




Ref. *S. Granier et al., Nature, 485 (2012) 400-404.*
H. Wu et al., Nature, 485 (2012) 327-332.

Molecular recognition of opioid ligand-receptor interactions: *In silico* research.

Endomorphin-1: Tyr-Pro-Trp-Phe-NH_2



Opioid peptide	Message			Address
	Pharm	Spacer	Pharm	
Endomorphin-1	Tyr	Pro	Trp	Phe-NH_2
Dynorphin(1-8)	Tyr	Gly-Gly	Phe	Leu-Arg-Arg-Ile
Leu-enkephalin	Tyr	Gly-Gly	Phe	Leu-NH_2

Two types of endogenous peptides – message sequence:

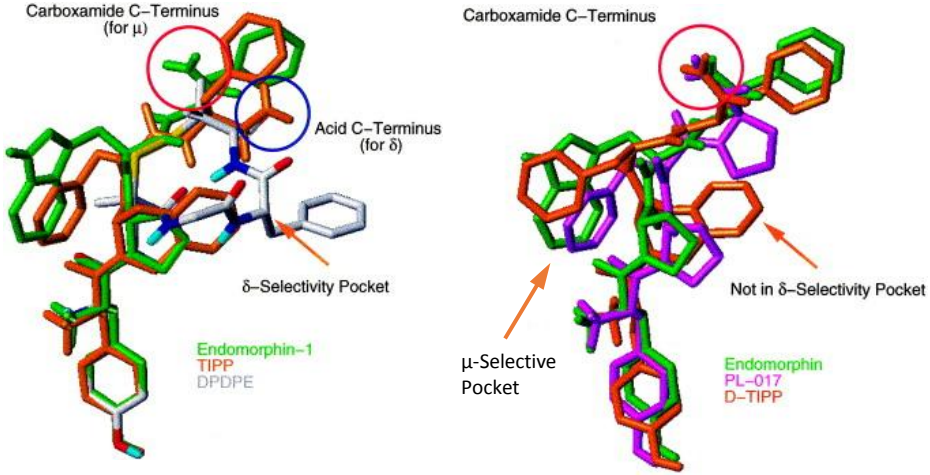
1. Tyr-Gly-Gly-Phe
   Enkephalins, endorphins, dynorphins
2. Tyr-Pro-Phe/Trp
   Endomorphins

Structural constraints in message sequence are key factor for opioid activity

Ref. *X. Liu et al., Bioorg Med Chem Lett, 19 (2009) 5387-5391.*

Structure-activity/selectivity relationship for opioid receptors.

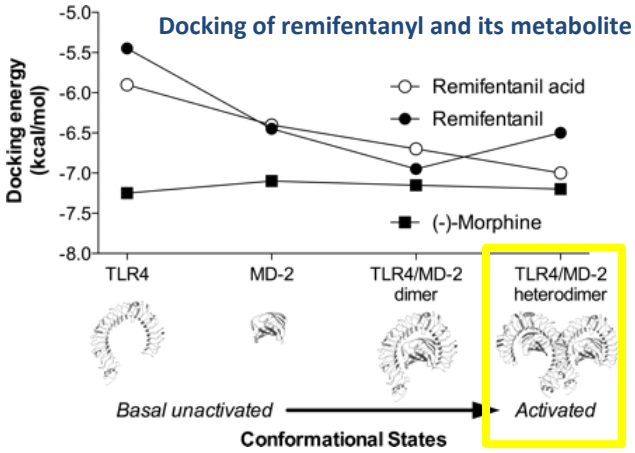
1. Structural characteristics determine activity



Dual μ-agonist/δ-antagonist profile of TIPP-NH_2

2. Interplay with other receptors

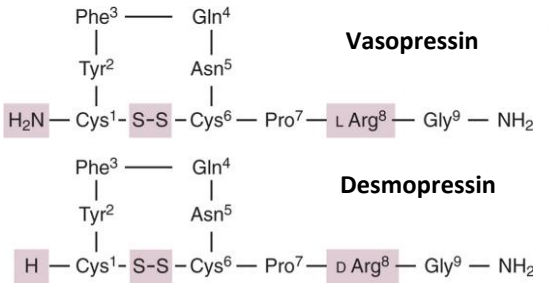
- Binding to other receptors, *e.g.* TLR4/MD2



Altered opioid reward behavior

- Heterodimerization:
  - ✓ Change the endocytosis/recycling kinetics receptor
  - ✓ Allosterically enhancment of ligand binding capacities of the other partner
  - ✓ Functional modulation signaling pathways

3. Bioavailibility of peptide therapeutics

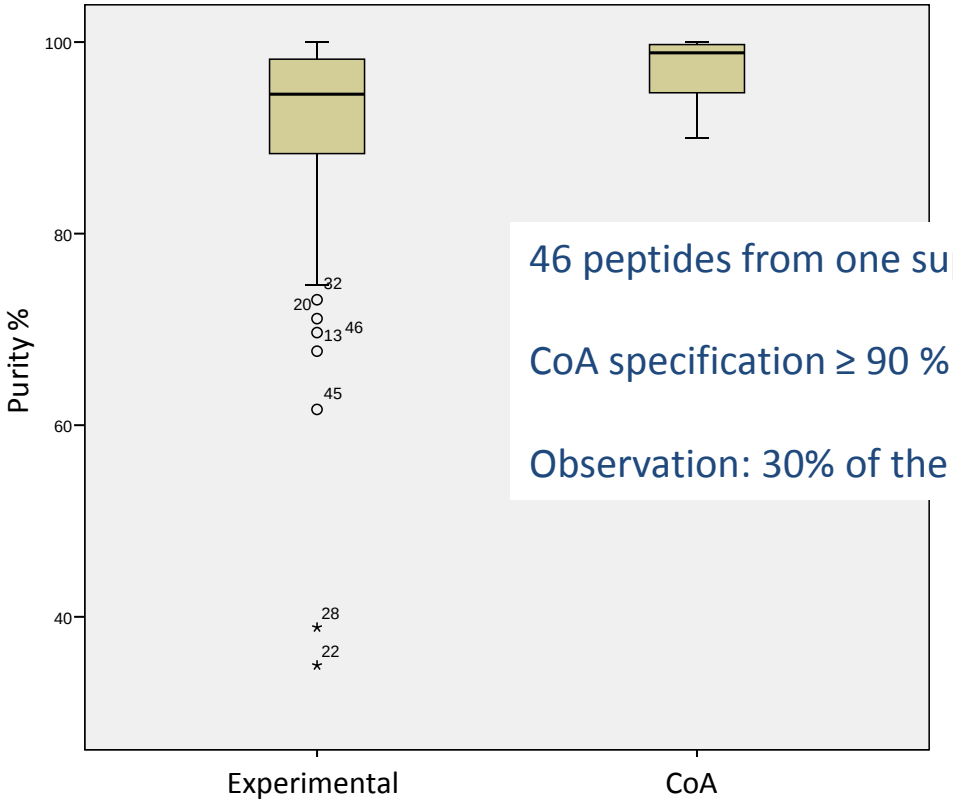


enhanced membrane permeation and enzymatic stability

Ref. *B.L. Podlogar et al.,FEBS Lett, 439 (1998) 13-20. M.R. Hutchinson et al., J Neurosci, 32 (2012) 11187-11200.*

In vitro opioid receptor binding assays: peptide purity level.

INTRODUCTION
IN-SILICO
IN VITRO
EX VIVO
IN VIVO
CONCLUSIONS



46 peptides from one supplier (PPR)

CoA specification ≥ 90 % HPLC purity

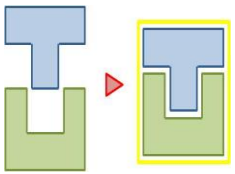
Observation: 30% of the peptides < 90 %

Also: 1 peptide from ≠ suppliers: problematic!

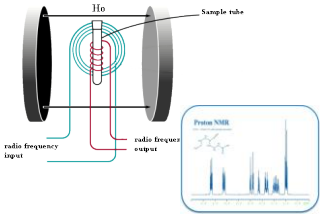
! Peptide quality: not assured/maintained !

Ref. *Van Dorpe S. et. al., J Bioanal Biomed, 2011, doi: 10.4172/1948-593X.S6-003.*
B. De Spiegeleer et al., Anal Biochem, 376 (2008) 229-234.

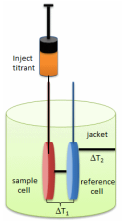
IN VITRO



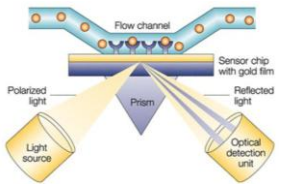
RLB



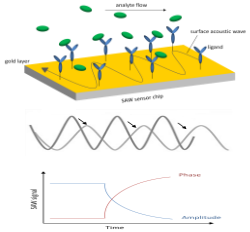
NMR



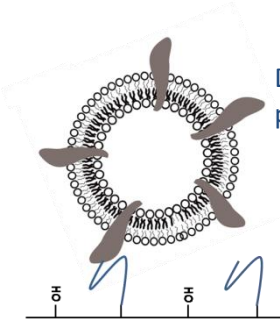
ITC



SPR



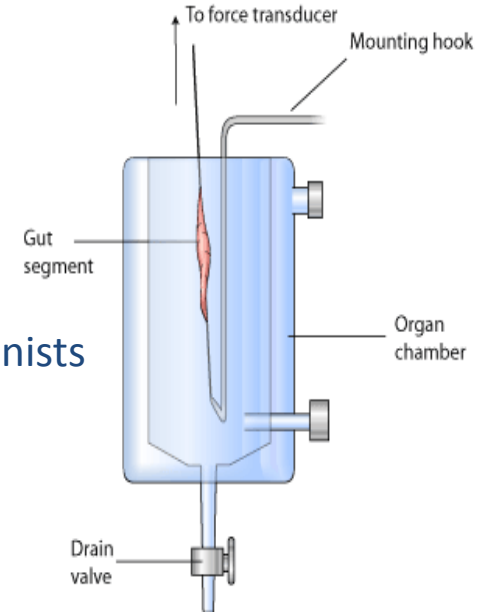
SAW

	RLB	NMR	ITC	SPR	SAW
Advantages	No immobilization Specific binding	Structure determination at atomical resolution Identification ligand-binding site	Label-free and no immobilization Ligand or protein size – no limitation All binding parameters in one experiment	Real time Label-free Higly sensitive	
Disadvantages	Labelling	Relatively poor sensitivity Upper limit for protein size	Requires relatively high [sample]	Immobilization Non-specific binding Mass of the analyte	
Developments	New RLB method to measure the concentration of non-radiolabeled drugs in the brain (*ex vivo*) *G. P. Hussmann, JPET, 2012*	Characterization of stability of backbone hydrogen (using a newly developed pressure cell Nisius and Grzesiek) *L. Nisius, Nature, 2012*	Nano ITC, *e.g.* analysis of Binding Organic Compounds to Nanoparticles by Isothermal Titration Calorimetry	Real-time kinetic analysis of K_D-value for label-free therapeutic antibody on native cell surface antigens	
Application	Investigation supraspinal cross-talk between MOR and DOR: enhanced sensitivity to anti-nociception induced by MOR agonists *J.J. Ballesta, BJP, 2012*	Binding characterization of linear, cyclic monomer and dimer of enkephalin *D.H. Kim, BKCS, 2012*	Complexation of the opioid peptide tynorphin and dipeptidyl peptidase III (ΔS dominated process) *(GA. Bezerra, PNAS, 2011)*	 DOR membrane preparations Immobilized opioid peptide	

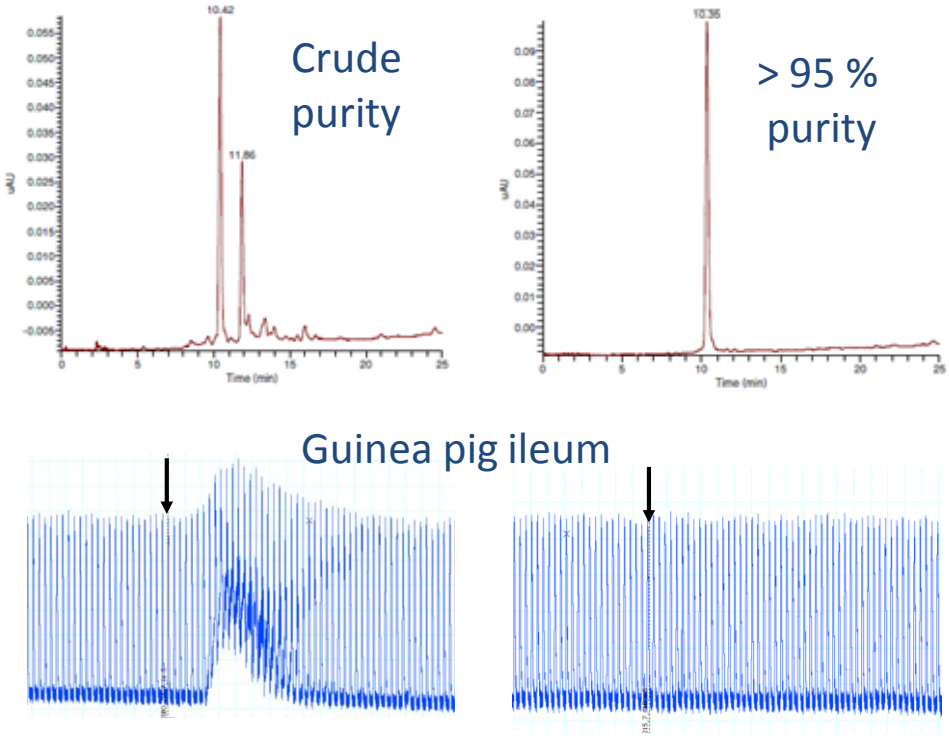
Tissue organ bath (TOB) experiments: "Golden standard"

INTRODUCTION
IN-SILICO
IN VITRO
EX VIVO
IN VIVO
CONCLUSIONS

- **Isolated tissues for opioid activity**:
  - ✓ Mouse vas deferens (δ >> μ > κ)
  - ✓ Guinea pig ileum (μ > κ >> δ)
- **Advantages**:
  - ✓ Relatively small amount of test material
  - ✓ Tissue responses (incl. adsorption, penetration, metabolization)
  - ✓ Identification receptor target using antagonists
- **Disadvantages**:
  - ✓ Non-specific binding
  - ✓ Adsorption to glass
- **Advances – mimicing *in-vivo* functionality**:
  - ✓ Bi-ventricular working heart system
  - ✓ Whole lung decellularization/recellularization chamber for regenerative medicine



Impurity profile of peptides



Stability profile of peptides.

Specification	SBO121	SBO215_7	SBO397
Reference sample			
Peptide purity	100 %	97.4 %	100 %
$n_{impurity\ peaks}$	0	1	0
Level max impurity	N/A	2.6 %	N/A
Test sample			
Recovery main peak	61.3 %	26.3 %	92.3 %
$n_{impurity\ peaks}$	0	3	0
Level max impurity	N/A	71.4 %	N/A
Mass balance	61.3 %	99.7 %	92.3 %
Conclusion	Adsorption	Degradation	Stable

N/A: not applicable, as only one peak is observed due to the main peak present
Test sample: 10 min exposure to tissue organ bath with isolated guinea pig ileum

QUALITY OF PEPTIDES IS AN IMPORTANT ASPECT

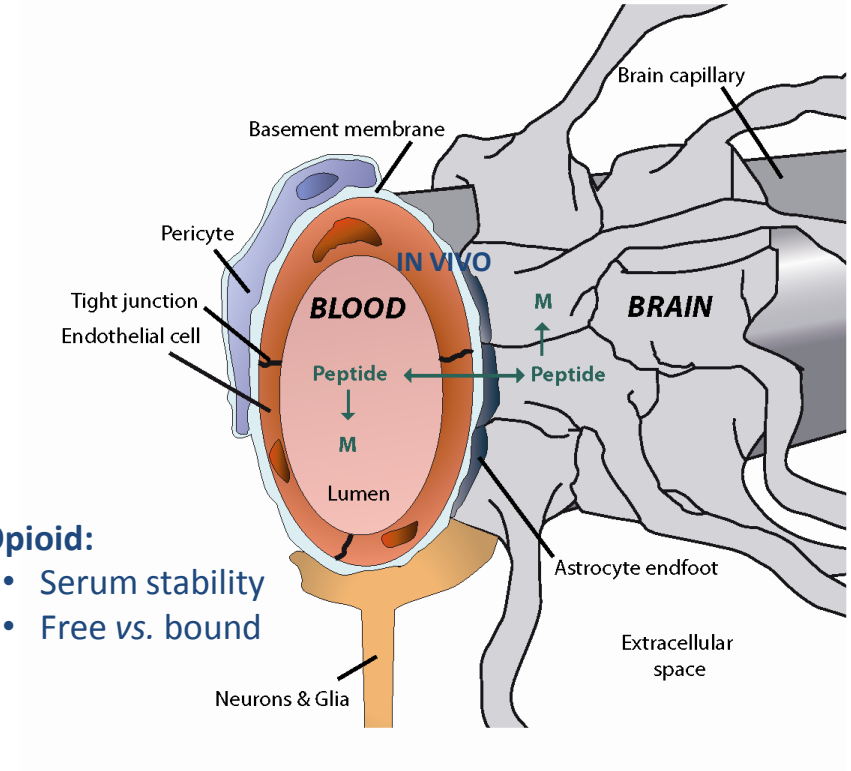
TO AVOID FALSE FUNCTIONALITY CONCLUSIONS

Ref. *M. Verbeken et al., Anal Biochem, 421 (2011) 547-555.*

Pain managment within the CNS: Opioid peptides should cross the BBB.

MOR main target pain management: Pharmacokinetic properties

Opioid:
- Serum stability
- Free *vs.* bound



BBB penetration mechanisms:
- Influx ($K_{in,}$ PS, P)
- Efflux ($K_{out,}$ $t_{1/2\ brain}$)

Brain distribution mechanisms:
- Metabolic clearance ($t_{1/2\ brain}$)
- Plasma protein binding (Free *vs.* bound)
- Non specific binding
- Clearance to ECF and CSF

BBB kinetics *vs.* central nervous system functionality: $BBB^{+/-}$ *vs.* $CNS^{+/-}$

Ref. *S. Van Dorpe et al., Brain Struct Funct, 217 (2012) 687-718.*

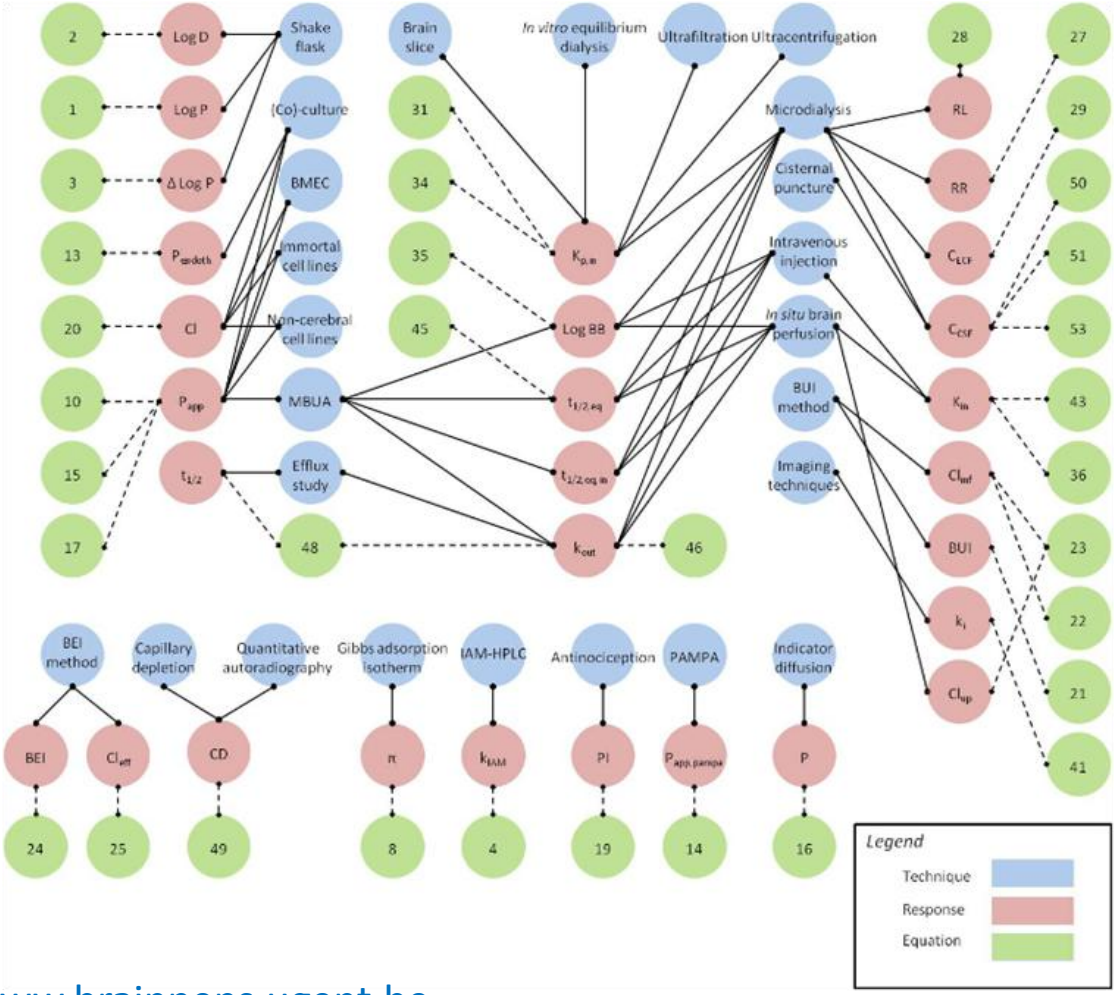
UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

Penetration =
Permeation (rate): P_{app}, K_{in}
+
Distribution (extent): B/P, K_p

1 technique: ≠ responses
1 response: ≠ techniques

Correlation

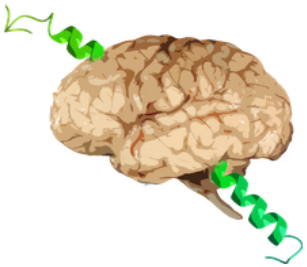


Brainpeps database: www.brainpeps.ugent.be

Ref. *S. Van Dorpe et al., Brain Struct Funct, 217 (2012) 687-718.*

IN VIVO

BRAINPEPS



Brainpeps® is a resource of blood
University. Based upon your inpu
physicochemical properties and re
the blood-brain barrier peptide d
correlated to each other.

Reference: BIBTEX

Please indicate first which input you want to use for your search in t

Dermorphin Search

Search through all structure / peptide properties.
e.g. Peptide name, 1 letter sequence, ...

Structure	Peptide name / Synonym(s)	1-letter code	3-letter code
Method	Intravenous injection (multiple time regression) Efflux study (ICV) Immortal cell lines Brain slice Ultracentrifugation BUI method Capillary depletion IAM-HPLC Indicator diffusion	BMEC Shake flask Non-cerebral cell lines In vitro equilibrium dialysis Microdialysis Imaging techniques Quantitative autoradiography Antinociception HPLC	In situ brain perfusion (Co)-culture MBUA Ultrafiltration Cisternal puncture BEI method Gibbs adsorption isotherm PAMPA
Response	PI $t_{1/2,\ brain}$ CD (brain/serum) Log D P_{endoth} $K_{p,\ in}$ $t_{1/2,\ eq,\ in}$ C_{ECF} BUI BEI k_{IAM} V_i CD (parenchyma/serum)	K_{in} $t_{1/2,\ serum}$ CD (capillary/serum) Log P CI Log BB RL C_{CSF} k_i CI_{eff} $P_{app,\ pampa}$ % parenchyma	PS % injected dose k_{out} Δ Log P P_{app} $t_{1/2,\ eq}$ RR CI_{inf} CI_{up} π P % capillary
Physicochemical properties	Log P Number Amino Acid Residues	pI	Mw
Literature	Author Title	Year	Journal

Citation

If you are using or downloading data from the database, please cite "Van Dorpe S., Bronselaer A., Nielandt J., Stalmans S., Wynendaele E., Audenaert K., Van De Wiele C., Burvenich C., Peremans K., Hsuchou H., De Tré G., De Spiegeleer B. *Brainpeps: The blood-brain barrier peptide database*. Brain Structure and Function, 217 (3), 687-718".

General Information

- Data submission to Brainpeps
- Contact us

Brainpeps database: www.brainpeps.ugent.be

Ref. *S. Van Dorpe et al., Brain Struct Funct, 217 (2012) 687-718.*

Drugability of opioid peptides: one global score which includes different characteristics.

Drugability reflected in a desirability value:

- **Receptor affinity**: K_D
- **Metabolic stability**: plasma *vs.* brain
- **BBB behavior**: influx *vs.* efflux

$$d(Y) = \frac{Y_i - 0.9Y_{min}}{1.1Y_{max} - 0.9Y_{min}}$$

$$d(Y) = \frac{1.1Y_{max} - Y_i}{1.1Y_{max} - 0.9Y_{min}}$$

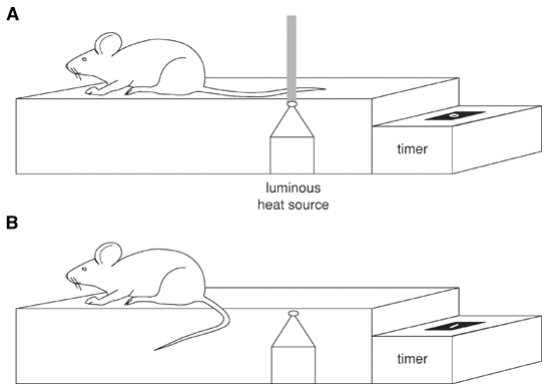
$$D = \sqrt[n]{\prod_{i=1}^{n} d_i^{p_i}}$$

Peptide	D-value	Ranking
SB Aba	**0.956**	1
P41	0.929	2
P42	0.867	3
Dermorphin	0.711	4
EM-1	0.566	5
EM-2	0.551	6
TAPP	0.549	7
DAMGO	0.486	8
CTOP	0.448	9
TAPS	0.422	10
P43	0.224	11
CTAP	**0.214**	12

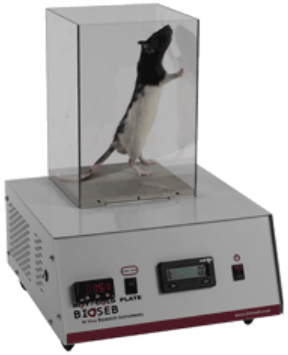
Ref. *S. Van Dorpe et al., J Pept Sci, 17 (2011) 398-404.*

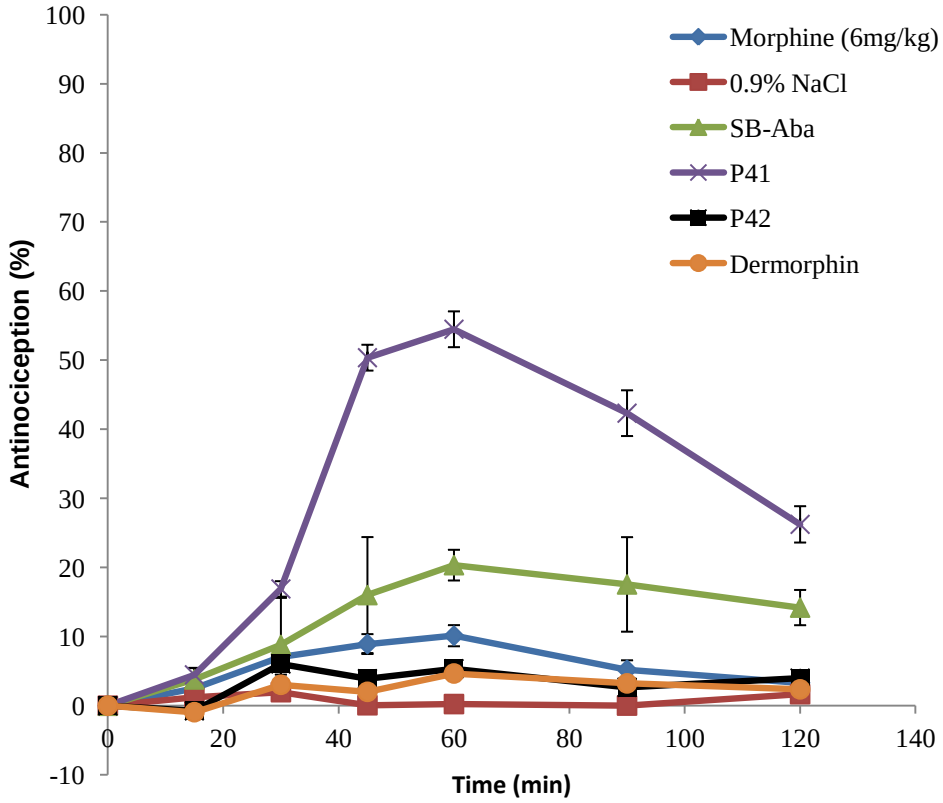
Determination of the overall antinociceptive activity of opioid peptides.

Tail-flick



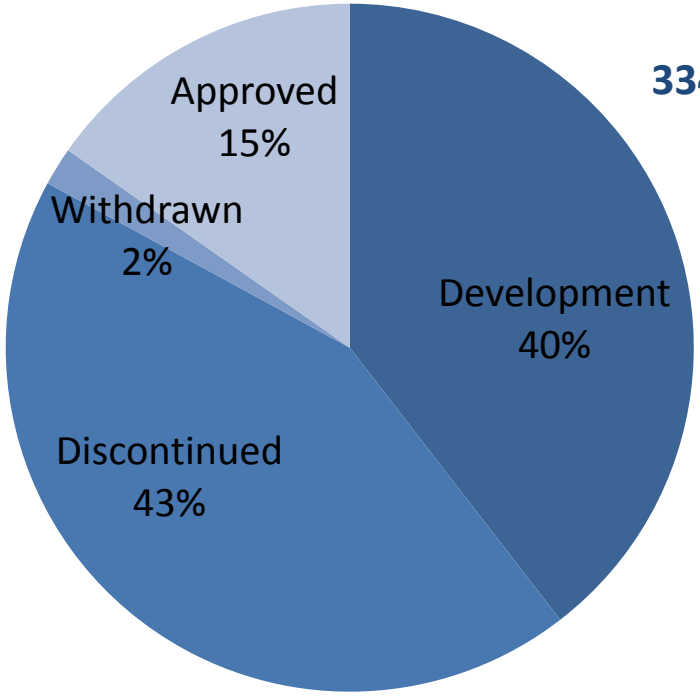
Hot plate test





Ref. *A. Novoa et al., J Med Chem, 2012, submitted.*

1. STATUS PEPTIDES (2010)



334 therapeutic peptides

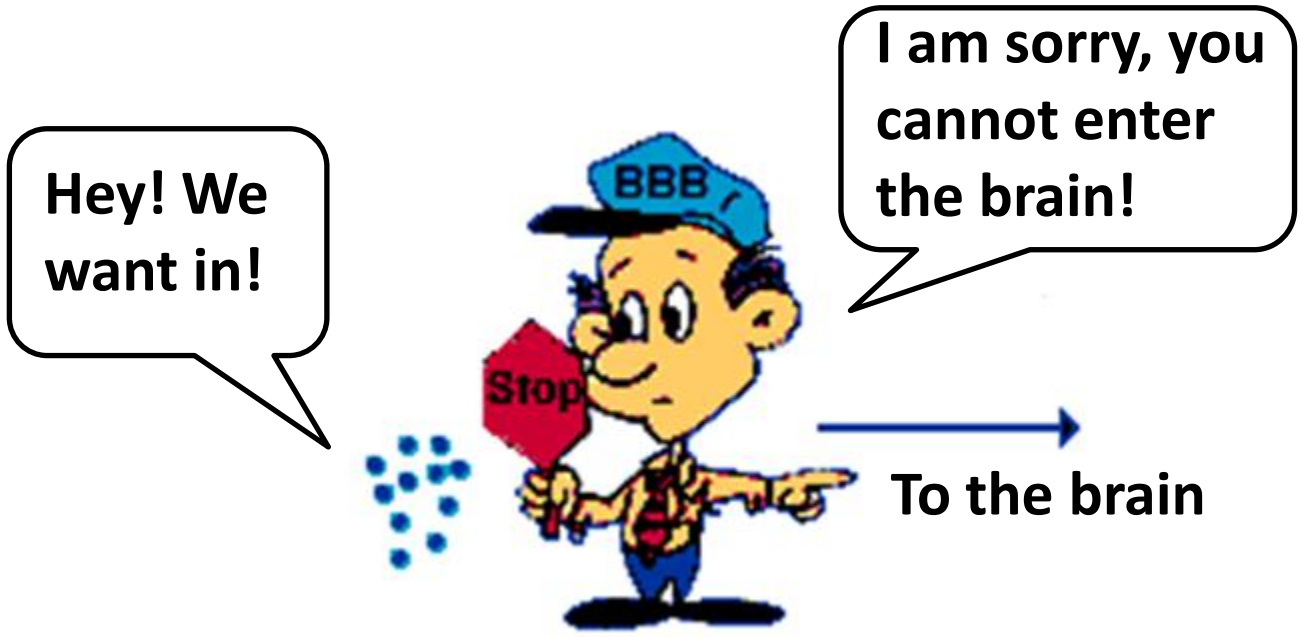
Challenge for opioid peptides:
analgesic activity lacking the dependence liability

2. TO STORE IN THE HIPPOCAMPUS (long-term memory)

- Dissection of pharmacological roles and interplay of “systems” (pain-immune)
- Receptor affinity and selectivity (biological activity): message-address related, as well as metabolic stability and BBB behavior
  => DRUGABILITY
- Quality and stability of peptides is an important aspect to avoid false functionality conclusions in opioid-peptide research

INTRODUCTION
IN-SILICO
IN VITRO
EX VIVO
IN VIVO
CONCLUSIONS

THANKS FOR YOUR ATTENTION



http://faculty.washington.edu/chudler/cart.html